

Figure 1

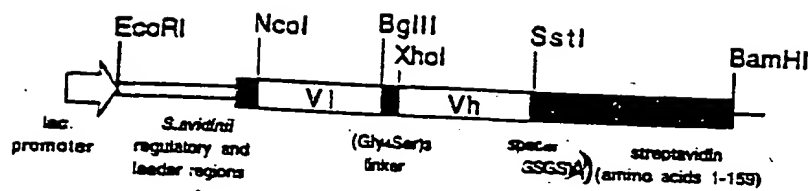


Figure 2

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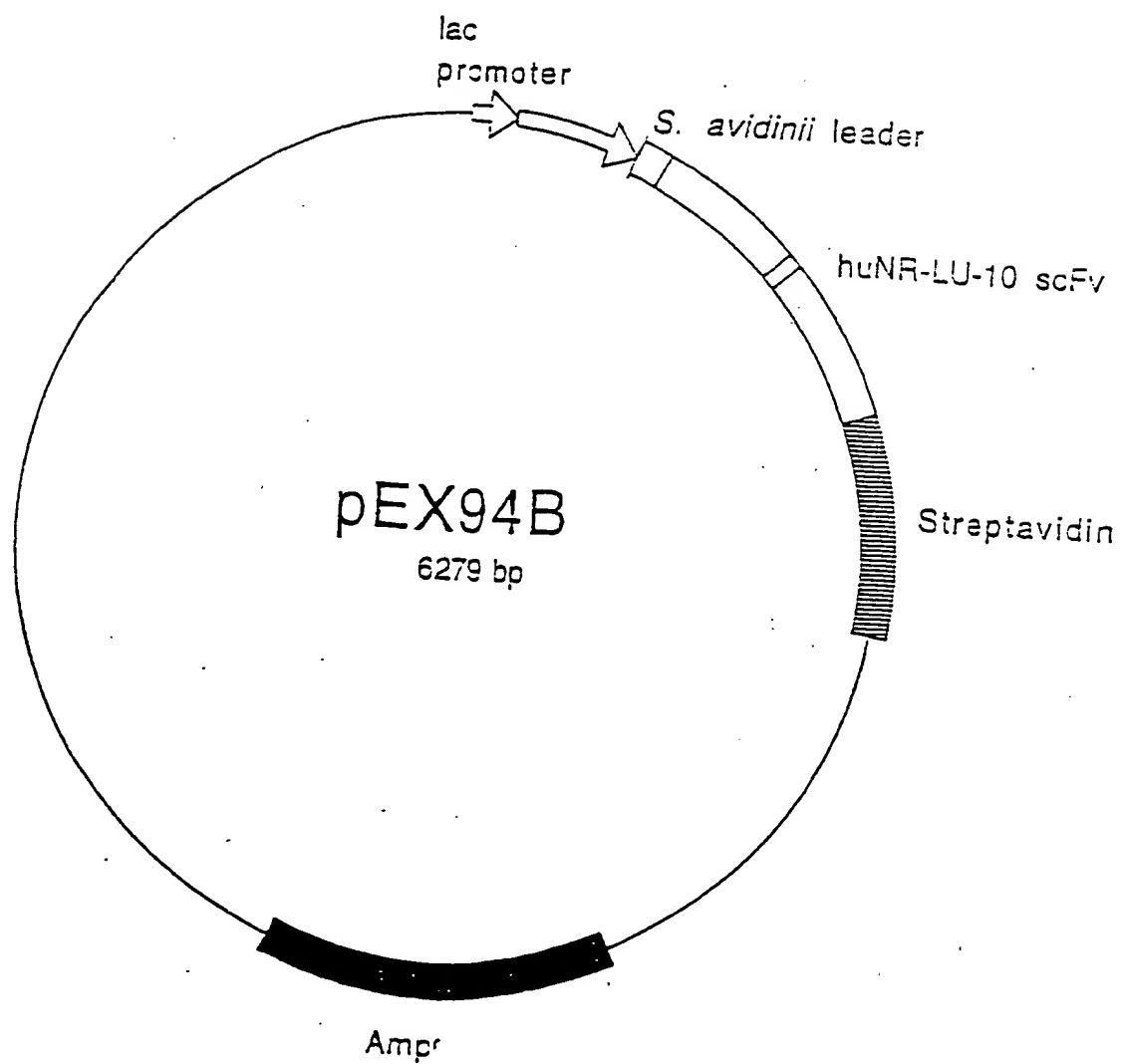


Figure 3

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Signal  
Sequence →

```

1      5'  CGCTCCGTCGCGCGCGCGGCAACAACCTAGCGAGTATTTTCGTGTCTCAG
          -20
30  Met Arg Lys Ile Val Val Ala Ala Ile Ala Val Ser Leu Thr Thr
    ATG CGC AAG ATC GTC GTT GCA GCC ATC GCC GTT TCC CTC ACC ACC
    Met
95  Val Ser Ile Thr Ala Ser Ala Ser Ala Asp Pro Ser Lys Asp Ser
    GTC TCG ATT ACC GCC AGC GCT TCG GCA GAC CCC TCC AAG GAC TCG
          10
140 Lys Ala Glu Val Ser Ala Ala Glu Ala Gly Ile Thr Gly Thr Trp
    AGG GCC CAG GTC TCG GCC GCC GAG GCC GCC ATC ACC GGC ACC TGG
          20
185 Tyr Asn Glu Leu Gly Ser Thr Phe Ile Val Thr Ala Gly Ala Asp
    TAC AAC CAG CTC GGC TCG ACC TTC ATC GTG ACC GCG GGC GGC GAC
          30
230 Gly Ala Leu Thr Gly Thr Tyr Glu Ser Ala Val Gly Asn Ala Glu
    GGC GCC CTC ACC GGA ACC TAC GAG TCG GCC GTC GCG AAC GCC GAG
          40
275 Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser Ala Pro Ala Thr
    AGC CGC TAC CTC CTC ACC GGT GGT TAC GAC AGC GCC GCG GGC ACC
          50
320 Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala Trp Lys Asn
    GAC GGC AGC GGC ACC GCC CTC GGT TGG ACC GTC GCC TCG AAG AAT
          60
365 Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly Glu Tyr
    AAC TAC CGC AAC GCC CAC TCG GCC ACC ACC TCG AGC GGC CAG TAC
          70
410 Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Glu Trp Leu Leu Thr
    GTC GGC GGC GCC GAG GCC AGC ATC AAC ACC CAG TGG CTC CTC ACC
          80
455 Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
    TCC GCC ACC ACC GAG GCC AAC GCC TGG AAG TCC ACC CTC GTC GGC
          90
500 His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Ser Ile Asp
    CAC GAC ACC TTC ACC AAG GTG AAG CCG TCG GCC GCC TCC ATC GAC
          100
545 Ala Ala Lys Lys Ala Gly Val Asn Asn Gly Asn Pro Leu Asp Ala
    GCG GCC AAG AAG GCC GCC GTC AAC AAG GCC AAC CCG CTC GAC GCC
          110
          120
          130
          140
          150
          HincII
          Val Glu Glu Stop
590 GTT CAG CAG TAG TCGCGTCCCGGCAACCGCGCGGTGCGCGGACCTCGGCC 3'

```

Figure 4

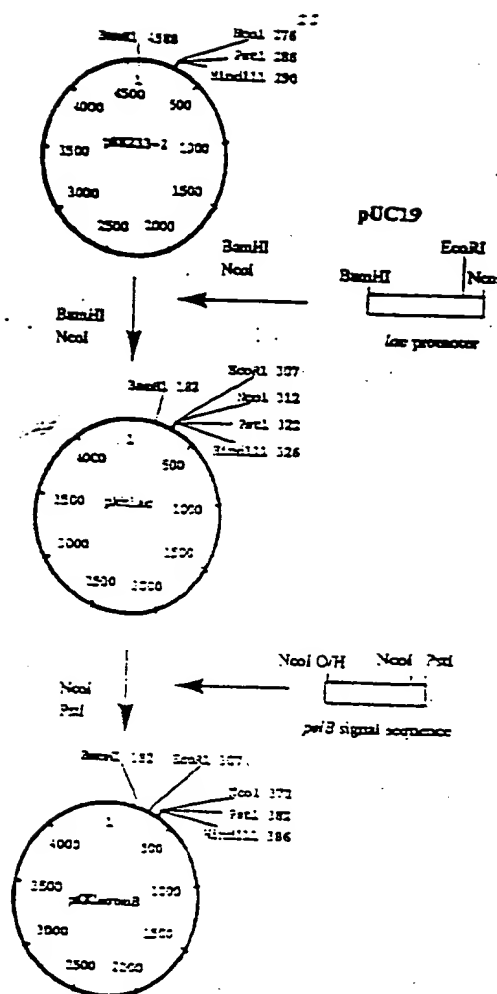


Figure 5

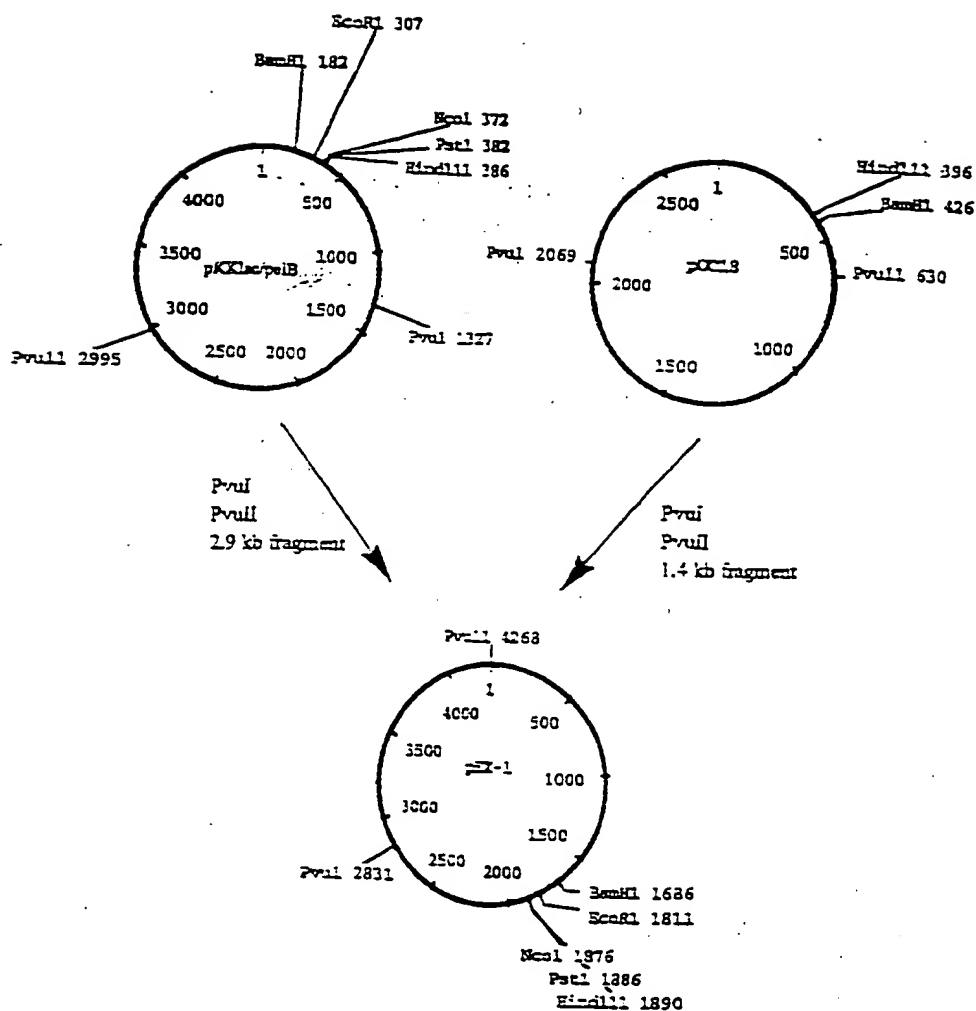


Figure 6

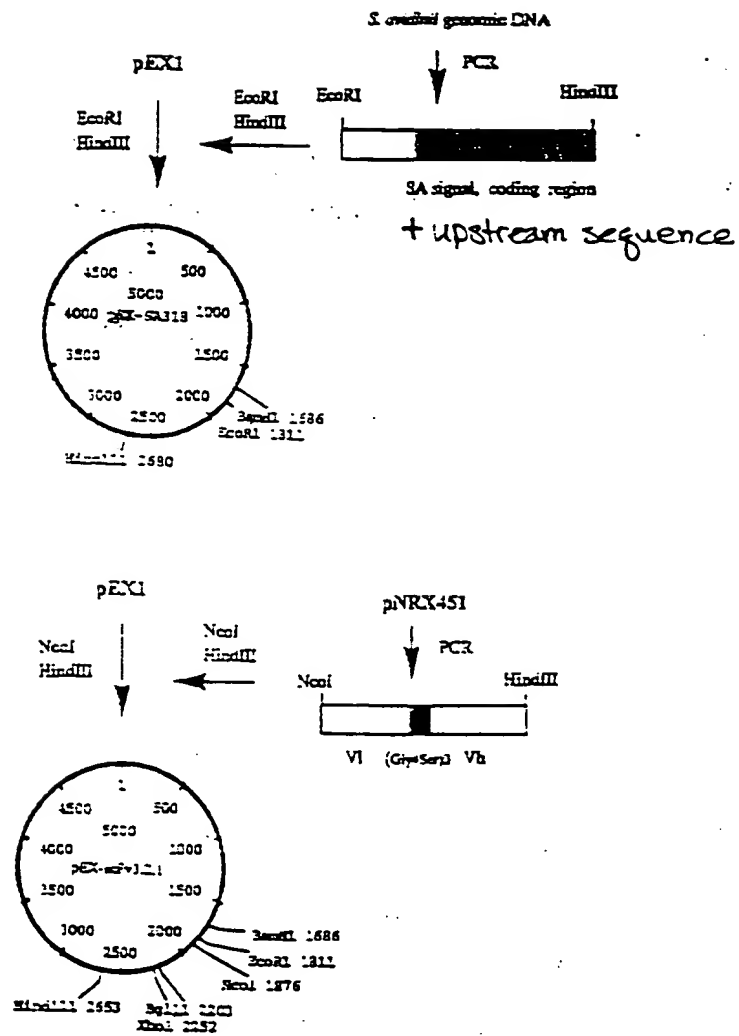


Figure 7

pEX94B

HindIII  
ScaI  
1.5 kb  
fragment

BamHI, fill-in  
HindIII

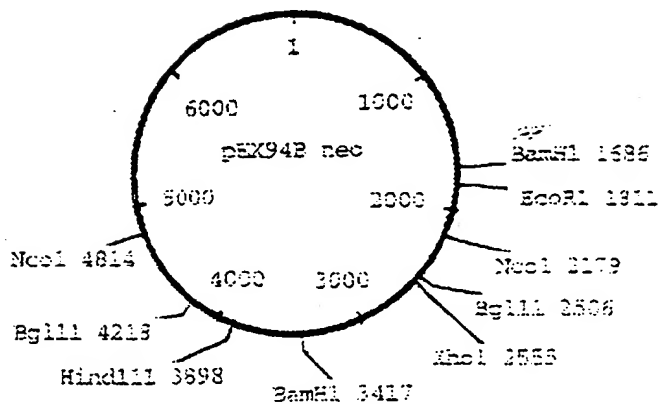
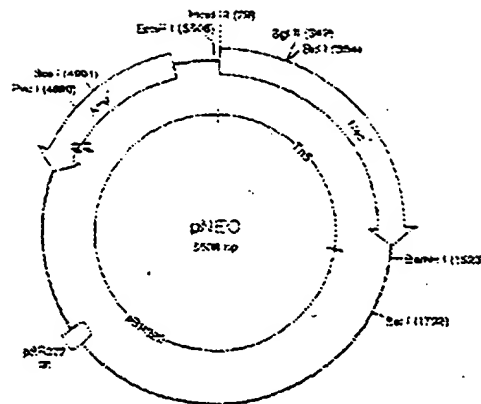


Figure 9

005090-0266360

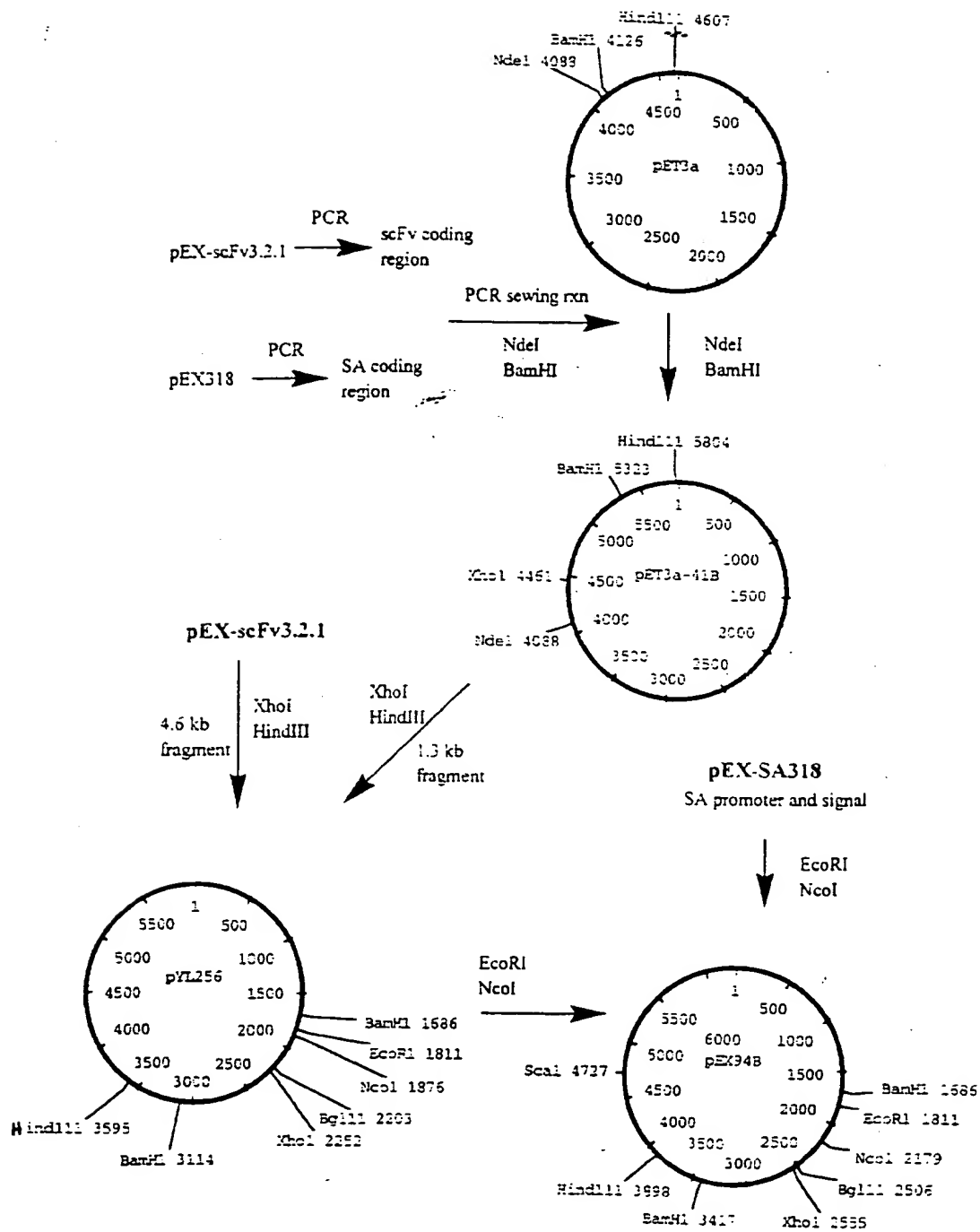


Figure 8

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**THE** **NEW** **YORK** **PUBLIC** **LIBRARY**

1 GAATTCACGAAGTAACCGACAGGACTCGGCCATTCTTTGGCCGAAATTCCTTTGCAGAAA  
61 ATGTTGTTGAGAAACCCCTCGATGGCTAGTACGATTACACCGAACATGTGCCCTTGGCAA  
121 CCAATCGACCCGACCTCGACTATCGAGTTCTGCGCCGCAAGACACATGCCGCACTGCTGT  
181 TTGTTACCGACACCGTCAGGTGCACGGCCGAGGTCAAAACCTGACGGGGGGGATACG  
241 GACGGCGCGATCGCAACAGCGCGCCCTCCGTCCCCCGCCGGGCAACACTAGGGAGTATTTTT  
301 CGTGTCTCAGTCGCGCAAGATCGTCTGTGACGCTTCGCCGTTTCCCTGACCACGGTCTC  
M R K I V V A A I A V S L T T V S  
361 GATTAACGGCATGGCTGACATCGAGATGACTCAGTCTCCATCGTCTTTGTCTGCCCTCTGT  
121 I T A M A A D I Q M T Q S P S S L S A S V  
signal peptidase  
421 GGGAGACAGAGTCACCATCCTTTGTCTGGGCTAGTCAGGGCATTAGAGGTAATTTAGACTG  
141 G D R V T I T C R A S Q G I R G N L D W  
481 GTATCAGCGAGAACTTGGTAAGGACCGGAACTCTTAATCTACTCCACATCCAAATTTAAA  
161 Y Q Q K P G K G P K L L I Y S T S N L N  
541 TTCTGGTGTCCCATCGAGTTTCAGTGGCAGTGGGTTCTGGGTACAGTTATCTCTCACCAT  
181 S G V F S R F S G S G S G S D Y T L T I  
601 CAGCAGCCCTTCAGCCTGAAGATTTCGCACTATTACTGTCTACAGCGTAATGCGTATCC  
201 S S L Q P E D F A T Y Y C L Q R N A Y P  
661 GTACACCTTCGGAACAGGGACCGAGCTGGAGATCAGATCTCTGGTGGCGGTGGCTCGGG  
221 Y T F G Q G T K L S I K I S G G G G S G  
linker  
721 CGGTGGTGGGTCTGGGTGGCGGAGGCTCGAGCCAGGTTGAGCTGGTCCAGTCTGGGGCAGA  
241 G G G S G G G G S S Q V Q L V Q S G A E  
781 GGTGAAAAAGCCAGGGGCTCAGTCAAGGTGTCTGCAAGGCTTCTGGCTTCAACATTAA  
261 V K K P G A S V K V S C K A S G P N I K  
841 AGACACCTATATGCACTGGGTGAGGCGAGGCACTGGACAGGGCTGCACTGGATGGGAAG  
281 D T Y M H W V R Q A P G Q G L Q W M G R  
901 GATTGATCCTGCGAATGGTAATACTAAATCCGACCTGTCTTCCAGGGCAGGGTGACTAT  
301 I D P A N G N T K S D L S P Q G R V T I  
961 AACAGCAGACACGTTCCATTCACACAGCCTACATGGAACTCAGCAGCCTGAGGTCTGACGA  
321 T A D T S I N T A Y M E L S S L R S D D  
1021 CACTGGCGCTCTATTACTGTCTTAGAGAGTCTCCTACTGGGACGTGGTCTTTGGACTACTG  
341 T A V Y Y C S R E V L T G T W S L D Y W  
linker  
1081 GGGTCAGGAACCTTAGTCACCTCGAGCTCTGCTCTGGTTCGGGACCTCTCCAGGA

streptavidin  
regulatory  
region

streptavidin  
Signal sequence

 $V_L$  $\rightarrow V_H$

Figure 10 Cont.

361 G Q G T L V T V S S G S G S A D P S K D

1141 CTGGAGGCCCAGGTCTCGGCGCGAGGCCGCGATCAGCGGCTCTTGCTACACCGCT  
 381 S K A Q V S A A E A G I T G T W Y N Q L

1201 CGGCTCGACCTTCATCGTGACCGCGGCGCGACCGCGGCTGACCGGAACCTACGATC  
 401 G S T F I V T A G A D G A L T G T Y E S

1261 GGCGCTCGGCAACCGCGAGAGCGCGTACGTCCTGACCGCTCTGACGACAGCGCGCGCGC  
 421 A V G N A E S R Y V L T G R Y D S A P A

1321 CACCGACCGGAGCGCGACCGCGCTCTGCGCTGGACCGCTGGCGTGAAGAATACTACCGCA  
 441 T D G S G T A L G N T V A W K N N Y R N

1381 CGCGGCTCTCGCGACCGACGCTGGAGCGCGCTAGTACTCGCGCGCGCGGCGGCGAGGATCAA  
 461 A H S A T T N S G Q Y V G G A E A R I N

1441 CACCGAGTGGCTGCTGACCTCGCGGCGCGACCGAGCGCGACCGCTGGAAGTCCACGCTGGT  
 481 T Q W L L T S G T T E A N A W K S T L V

1501 CGGCGACGACACCTTCACCGAGGTGAAGCGCTCGCGCGCTCGATCGACCGCGCGAAGAA  
 501 G H D T F T R V K P S A A S I D A A K K

1561 GGCGGCGCTCAACAACCGCGACCGCTCGACCGCTTCAGCAGTAAGGATCC  
 521 A G V N N G N P L D A V Q Q \*

streptavidin  
1-159

GACATCGTGC TGTGCGCAGTC TCCAGCAATC CTGTCTGCAT CTCAGGGGA GAA  
 GGTACAATG CTGTCAGGG CCAGCTCAAG TGTAAGTAC ATCTGGT ACCAGCAGAA  
 GCCAGGATCC CCAAAC CTTGGATTGA TGCCACATCC AAGGCTT CTGGAGTCCC  
 TGCTCGCTTC AGGCGCAGTG GGTCTGGGAC CTCTACTCT CTCAATCA GCAGAGTGGA  
 GGCTGAAGAT GCTGCCACTT ATTACTGCCA GCAGTGGATT AGTAACCCAC CCACGTTCCG  
TGCTGGGACC AAGCTGGAGC TGAAGATCTC TGGTCTGGAA GGCAGCCCGG AAGCAGGTCT  
GTCTCCGGAC GCAGGTTCCG GCTCGAGCCA GGTTCAGCTG GTCCAGTCAG GGGCTGAGCT  
 GGTGAAGCCT GGGGCCTCAG TGAAGATGTC CTGCAAGGCT TCTGGCTACA CATTTACCAG  
 TTACAATATG CACTGGGTAA AGCAGACACC TGGACAGGGC CTGGAATGGA TTGGAGCTAT  
 TTATCCAGGA AATGGTGATA CTTCTACAA TCAGAAGTTC AAAGGCAAGG CCACATTGAC  
 TGCAGACAAA TCCTCCAGCA CAGCCTACAT GCAGCTCAG AGCCTGACAT CTGAGGACTC  
 TGCGGTCTAT TACTGTGCAA GAGCGCAATT ACGACCTAAC TACTGGTACT TCGATGTCTG  
 GGGCGCAGGG ACCACGGTCA CCGTGAGCTC TGGCTCTGGT TCGGCA GACC CCTCCAAGGA  
 CTCGAAGGCC CAGGTCTCGG CCGCCGAGGC CGGCATCACC GGCACCTGGT ACAACCAGCT  
 CGGCTCGACC TTCATCGTGA CCGCGGGCGC CGACGGCGCC CTGACCGGAA CCTACGAGTC  
 GCGCGTCGGC AACGCCGAGA GCCGCTACGT CCTGACCGGT CGTTACGACA GCGCCCCGGC  
 CACCGACGGC AGCGGCACCG CCCTCGGTTG GACGGTGGCC TGAAGAATA ACTACCGCAA  
 CGCCCACTCC GCGACCACGT GGAGCGGCCA GTACGTCCGC GCGCCGAGG CGAGGATCAA  
 CACCCAGTGG CTGCTGACCT CCGGCACCAC CGAGGCCAAC GCCTGGAAGT CCACGCTGGT  
 CGGCCACGAC ACCTTCACCA AGGTGAAGCC GTCCGCGGCC TCCATCGACG CGGCGAAGAA  
 GCGCGCGTC AACACGGCA ACCCGCTCGA CGCCGTTGAG CAGTAA

11A

Translation of B9E9pKOD scFvSA

Figure 11B

DIVLSQSPAIL SASPGEKVTM TCRASSSVSY MHWYQKPGS SPKPWYATS NLASGVPARF  
 SGSGSGTSYS LTISRVEAED AATFYCQQWI SNPTFGAGT KLELKIS GLE GSPEAGLSPD  
AGSGSQVQL VQSGAEL VKP GASVQMSCKA SGYTFTSYNM HWVKQTPGQG LEWIGATYPG  
 NGDTSYNOKF K GKATLTADK SSSTA YMQLS SLTSEDSAVY YCARAQLRPN YWYFDVWGAG  
 TTVTVS SGS SADPKDSKA QVSAAEAGT GTWYNQLGST FVTAGADGA LTGTYESAVG  
 NAESRYVLTG RYDSAPATDG SGTALGWTVA WQNNYRNAHS ATTWSGQYVG GAEARINTQW  
 LLTSGTTEAN AWKSTLVGHDTFTKVKPSAA SIDAACKAGV NNGNPLDAVQ Q\*

V<sub>L</sub>  
 pKOD  
 V<sub>H</sub>  
 LINKER 2  
 SA

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Figure 11C

E31-2-20 plasmid: NcoI-BamHI fragment containing B9E9 Vh- linker-VL-SA gene

1 CCATGGCTCAGGTTCCAGCTGGTCCAGTCAGGGGCTGAGCTGGTGAAGCCTGGGGCCTCAG  
1 M A Q V Q L V Q S G A E L V K P G A S V

61 TGAAGATGTCTGCAAGGCTTCTGGCTACACATTTACCAGTTACAATATGCACTGGGTAA  
21 K M S C K A S G Y T F T S Y N M H W V K

121 AGCAGACACCTGCACAGGGCCTGGAATGGAATGGAGCTATTTATCCAGGAAATGGTGATA  
41 Q T P Q Q G L E W I G A I Y P G N G D T

181 CTTCTTACAATCAGAGTTCAAGGCAAGGCCACATTGACTGCAGACAAATCCTCCAGCA  
61 S Y N Q K F K G K A T L T A D K S S S T

241 CAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTACTGTGCAA  
01 A Y M Q L S S L T S E D S A V Y Y C A R

301 GAGCGCAATTACGACCTAACTACTGGTACTTCCATGTCTGGGGCGCAGGGACCACGGTCA  
101 A Q L R P N Y W Y P D V W G A G T T V T

361 CCGTGAGCAAGATCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGTGGGTGGCGGGCGCT  
121 V S K I S G G G G S G G G S G G G S  
linker

421 CGGGTGGTGGTGGGTGGGGCGGGCGGGCTCCAGCGACATCGTGTCTGCGAGTCTCCAG  
141 G G G G S G G G G S S D I V L S Q S P A

481 CAATCCTGTCTGCATCTCCAGGGGAGAGGTCACAATGACTTGCAGGGCCAGCTCAAGTG  
161 I L S A S P G E K V T M T C R A S S S V

541 TAAGTTACATGCAGTGGTACCAGCAGAGCCAGGATCCTCCCCCAACCTGGATTATTAG  
181 S Y M H W Y Q Q K P G S S P K P W I Y A

601 CCACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACCT  
201 T S N L A S G V P A R F S G S G S G T S

661 CTTACTCTCTCACAATCAGCAGAGTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGC  
221 Y S L T I S R V E A E D A A T Y Y C Q Q

721 AGTGGATTAGTAACCCACCCACGTTCCGTGGTGGGACCAAGCTGGAGCTGAAGAGCTCTG  
241 W I S N P P T F G A G T K L E L K S S G

781 GCTCTGGTTCGGCAGACCCCTCCAAGGACTCGAAGGCCAGGTCTCGGCCCGGAGGCCG  
261 S G S A D P S K D S K A Q V S A A E A G  
linker

841 GCATCACCAGCACCTGGTACAACAGGCTCGGCTCGACCTTCATCGTGACCGCGGGCGCCG  
281 I T G T W Y N Q L G S T F I V T A G A D

901 ACGGCGCCCTGACCGGAACCTACGAGTCGGCCCTCGGCAACGCCGAGAGCCGCTACGTCC  
301 G A L T G T Y E S A V G N A E S R Y V L

961 TGACCGGTGGTTACGACAGCGCCCCCGGCAAGCGGACCGGCGGACCGCCCTCGGTTGGA  
321 T G R Y D S A P A T D G S G T A L G W T

005050-005050

Streptavidin

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1021 CCGTGGCCTGGAAGAATAACTACCGCAACGCCCACTCCGCGACCACGTGGAGCGGCCAGT  
 341 V A W K N N Y R N A H S A T T W S G Q Y  
 1081 ACGTCGGCCGGCGCGAGGCGAGGATCAACACCCAGTGGCTGCTGACCTCCGGCACCACCG  
 361 V G G A E A R I N T Q W L L T S G T T E  
 1141 AGGCCAACGCCTGGAAGTCCACGCTGGTCGGCCACGACACCTTCACCAAGGTGAAGCCGT  
 381 A N A W K S T L V G H D T F T K V K P S  
 1201 CCGCCGCCTCCATCGACGCGGCGAAGAAGGCCGGCGTCAACAACGGCAACCCGCTCGACG  
 401 A A S I D A A K K A G V N N G N P L D A  
 1261 CCGTTCAGCAGTAAGGATCC  
 421 V Q Q + G S

FIG. 11C CONTINUED

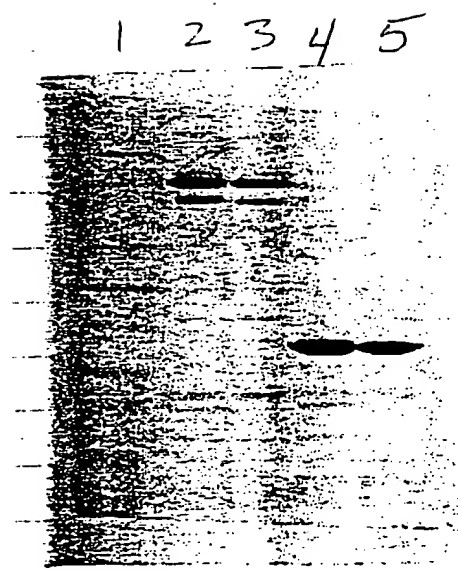


FIG. 12

005050-0266360

# Size Exclusion HPLC

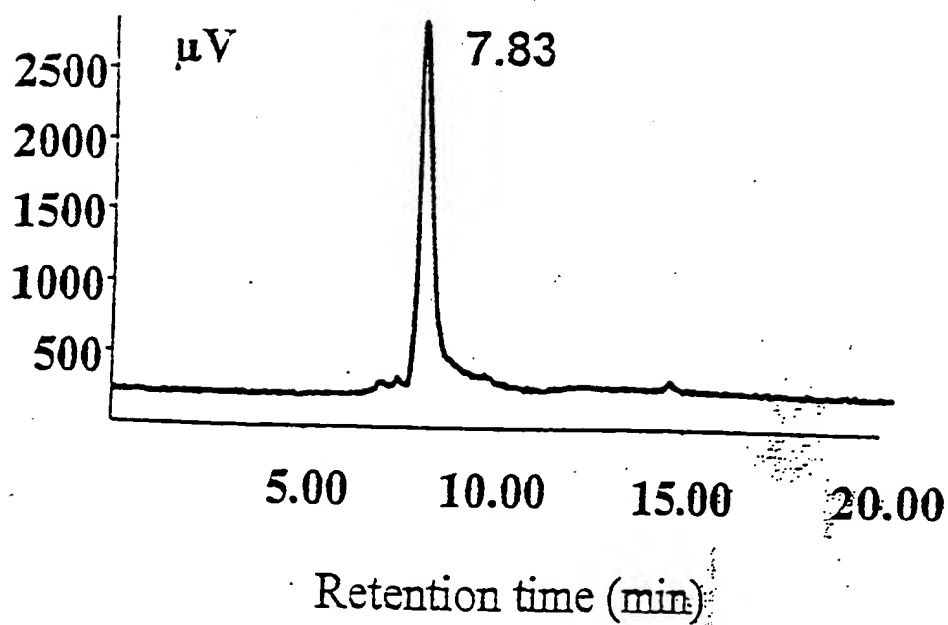


FIG. 13

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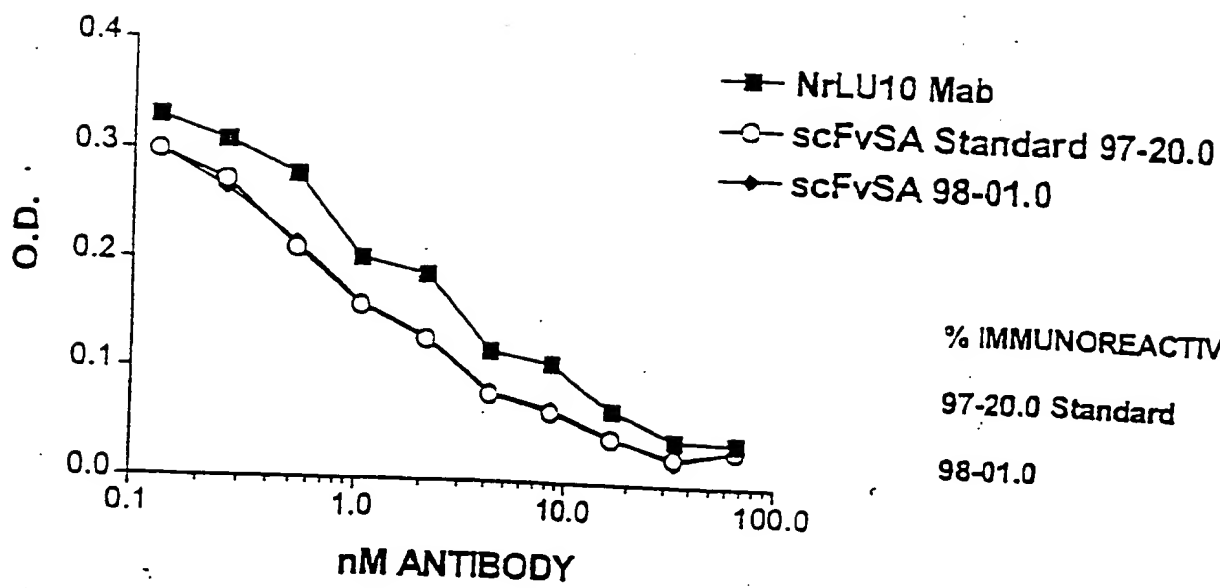


FIG. 14

005090-066560

Dissociation of DOTA-biotin  
from scFv-SA at 37 °C 1051-011

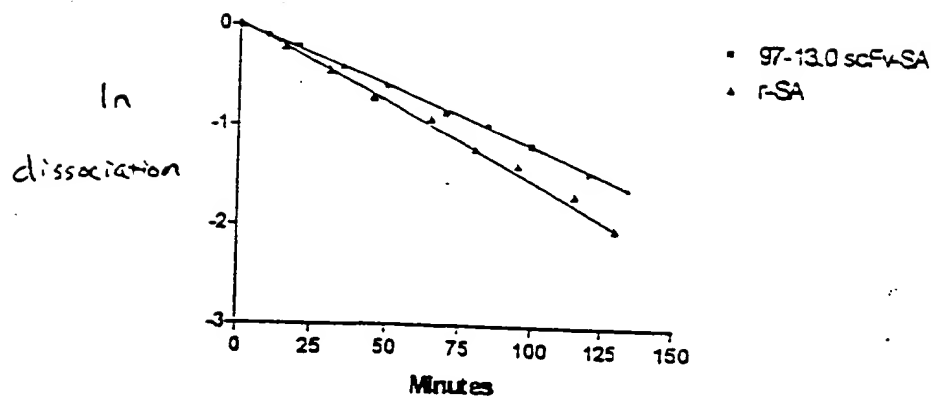


FIG. 15

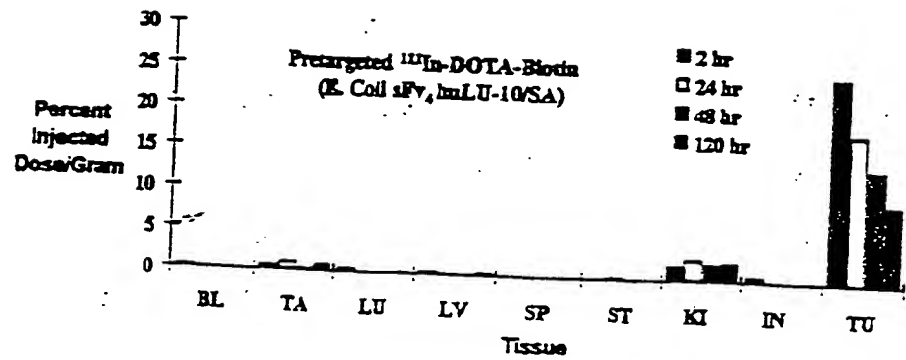


FIG. 16

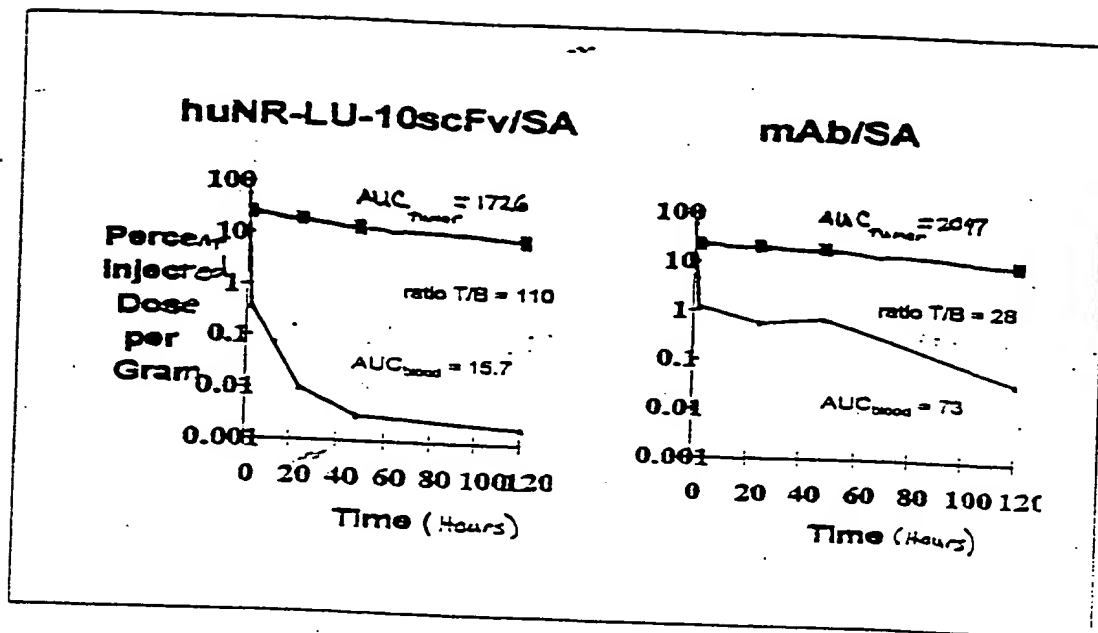


FIG. 17

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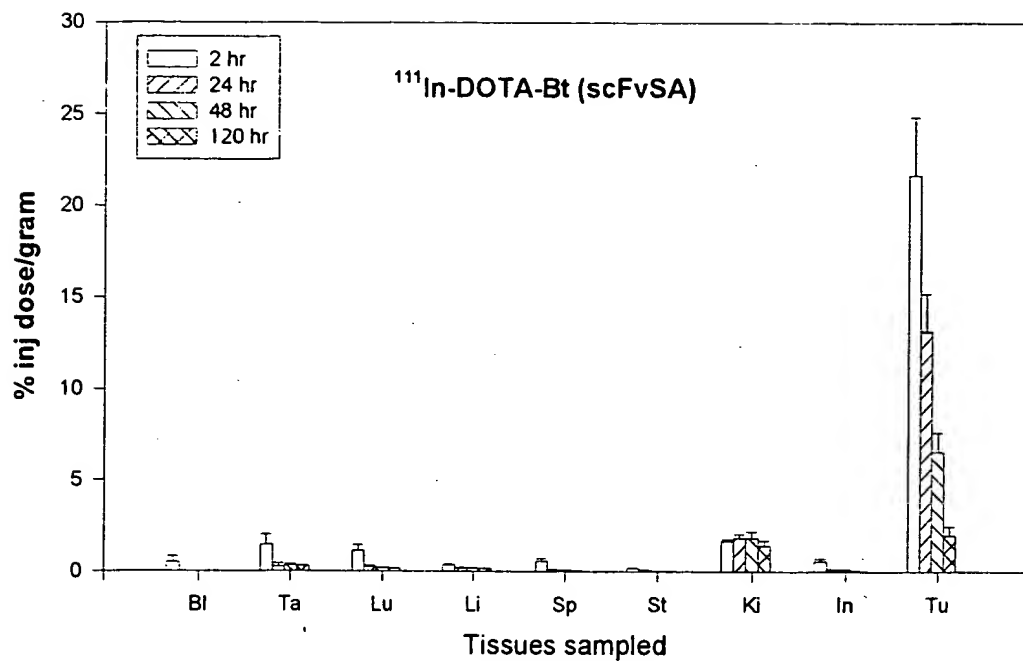


FIG. 18

Expression of scFvSA fusion proteins with or without FkpA.

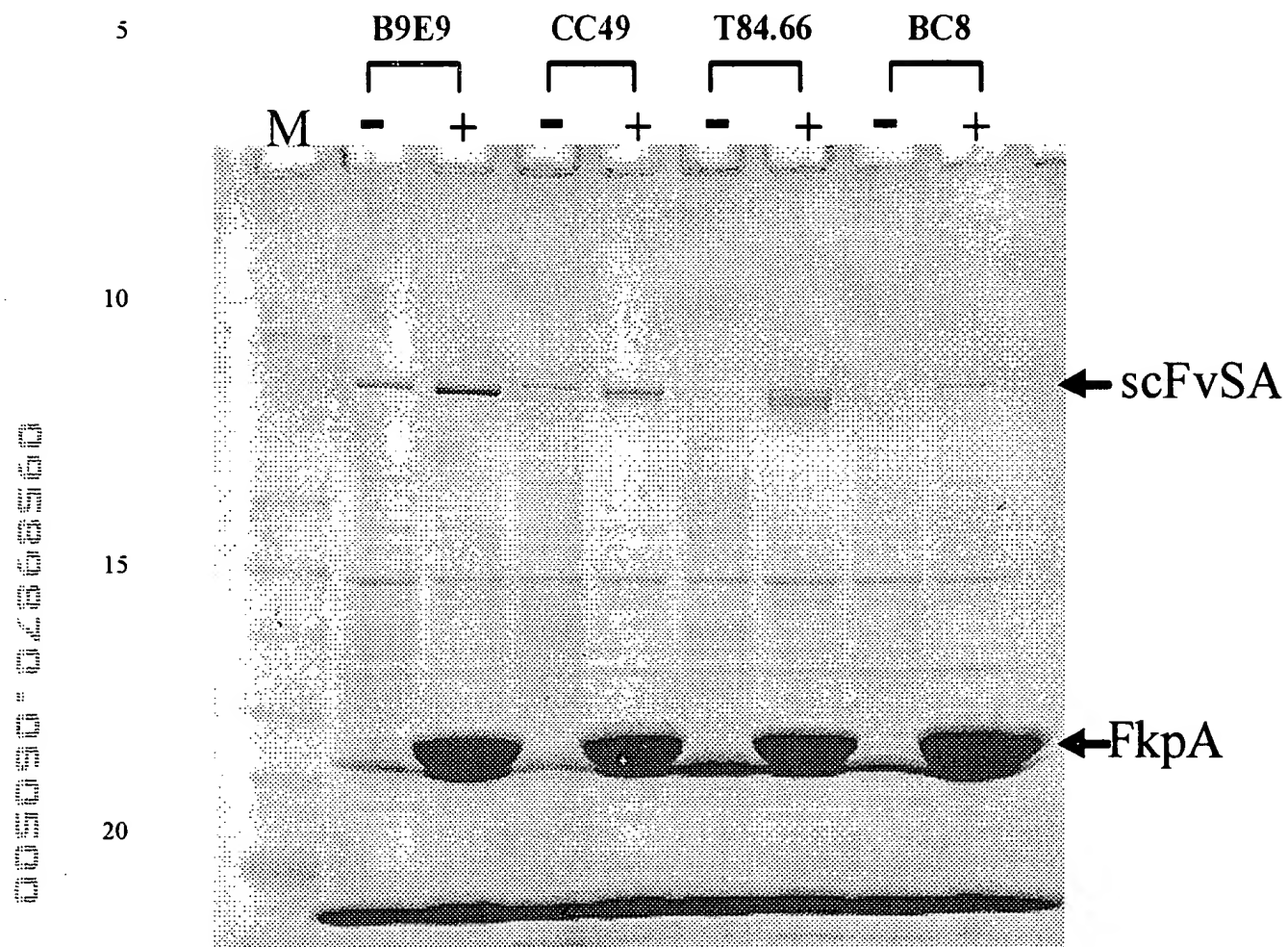


FIG. 19